Analysis of mass transfer of encapsulated porphyrins from PVA-based hydrogels: an experimental study on cylindrical gels and membranes

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Abstract: Poly (vinyl alcohol) (PVA) is a biocompatible polymer able to form a hydrogel network suitable for drug encapsulation and delivery. Porphyrins are used as photosensitizers in the photodynamic therapy of cancer. In the present work, we have studied experimentally the mass transfer phenomena of porphyrins from PVA-based hydrogels. The encapsulation of the porphyrin into the porous hydrogel structure has been accomplished by sorption. We have determined the influence of the shape of the gel (cylindrical- or membrane form) upon the release process. We have studied the diffusion process in distilled water but also in various physiological media. As the model porphyrin, TSPP (tetrakis 5,10,15,20 (p-sulphonato) phenyl porphyrin) has been used. Experiments have been conducted in batch vessels (Erlenmeyer flasks) but also in a vertically-agitated, 100 rpm (USP standard) thermostated controlled release apparatus. The release process of the porphyrin in the vertically agitated vessel has been monitored in a laminar flow environment (at small Reynolds numbers). Results have been compared between the Erlenmeyer flask and agitated vessel, in order to assign components of diffusive and convective mass transfer. A kinetic analysis has been performed.

Key-Words: convection, diffusion, mass transfer, porphyrin, hydrogel, photodynamic therapy, controlled release

1 Introduction

An important advance in the development of controlled delivery vehicles for drugs is represented by hydrogels. Hydrogels are three-dimensional polymer networks able to swell in water, whereby the degree of swelling depends strongly on the synthesis technique employed and on the type of polymer used [1-4]. The formation of physical or chemical bonds between polymer chains (crosslinks) increases the stability of the network, whereas the compatibility of the polymeric chains with water allows these materials to swell in water. Recently, hydrogels have been in the focus of research due to their ability for controlled release of pharmaceutical substances, including diffusion-mediated release, targeted release, swelling controlled drug release and bimodal release [5-8]. A special class of hydrogels, named "intelligent", due to their stimuli-responsive nature, has been used recently in a wide range of fields, such as pH- or temperature-triggered release of pharmaceuticals, medical devices, implants, artificial skin, wound dressing, contact lenses, biosensors and water decontamination devices. Intelligent hydrogels are able to release encapsulated drugs upon action of chemical (pH, electrolytes, and solvent action), physical factors (e.g. temperature) and magnetic fields (on-off release). New advances in biotechnology allowed the development of antigen-sensitive hydrogels, which are capable of releasing a specific therapeutic antibody in the presence of the required antigenic stimulus. Hydrogels based on poly (vinyl alcohol) are non-toxic, non-carcinogenic, biocompatible and biodegradable [1]. The PVA hydrogel has excellent properties regarding mechanical, water absorption and swelling characteristics. The crosslinking of PVA for the formation of hydrogels can take place by multiple methods, such as chemical (disadvantageous for use in medical applications because of toxicity issues) or physical (by freeze-thawing, leading to the formation of cryogels). The cryogenic crosslinking mechanism is preferred for biomedical applications, due to the fact that it enables the preparation of biodegradable and biocompatible hydrogels via formation of crystallites.

Porphyrins are a class of chemical compounds found ubiquitously in nature, from oxygen transport vehicles (hemoglobin), to compounds active in photosynthesis (chlorophyll), myoglobin (found in muscle), cytochromes (important respiratory role) and vitamins (for example, vitamin B12 and its cobalamine

derivatives with important biological function [9-12]. Recently, porphyrins have been applied to cancer photodynamic therapy (PDT, also known as photochemotherapy), a method based on applying a porphyrinic compound onto the tumour and then irradiating with a light source [13-20]. The porphyrin acts as a photosensitiser, transferring its energy to the oxygen found in tumoral tissue, generating singlet (radicalic) oxygen, which has the ability to oxidize tumour cells and also induce cell death (apoptosis). Until now, different porphyrins and porphyrin-related compounds have been tested for their antitumoral activity, e.g. phtalocyanines, purpurins, chlorin and porphycenes. Among these compounds, meso-substituted porphyrins have been preferred due to their high affinity to cancer cells and stronger photodynamic effects. By modifying the structure of the substituent, changes in solubility and also in the efficiency of uptake of the porphyrins by the cells can be effected. Thus the porphyrin TSPP (5,10,15,20-tetra (4-sulfophenyl) porphyrin), used in PDT, is water-soluble, while compounds such as TPP (5,10,15,20-tetra phenyl porphyrin) and TPyP (5,10,15,20-tetra p-pyridil porphyrin) are non water-soluble.

Currently, a strong tendency in cancer therapeutics is the use of controlled-release devices. Several methods have been used for the precise engineering of the release properties of drug delivery devices. PVA-based hydrogels are well suited for this purpose, due to their porous nanostructure, their biodegradability and biocompatibility. Release rate and total amount of drug released can be fine-tuned by engineering the physical structure of the gel (e.g. by varying the degree of crosslinking), creating asymmetries in the mesh structure of the membrane (e.g. by gradient crosslinking) or by creating devices with different geometries (e.g. multi-laminate devices, multi-layered tablets or nanoparticles).

Of particular interest is the characterization of mass transfer processes in such controlled release devices and the elucidation of the mechanism of mass transfer. The contributions of convective and diffusive mass transfer can be analysed under various conditions (temperature, pH, ionic strength of solution, agitation).

The present paper focuses on the determination of mass transfer properties of various release devices for porphyrins based PVA hydrogels. Hereby, mass transfer into distilled water and physiological media, in non-agitated vessels and in vertically agitated release devices and at room- (25°C) or body temperature (37°C) has been analyzed. Also, the release characteristic for these gels has been kinetically examined.

By considering the large array of applications of PVA hydrogels in biotechnology, advanced drug delivery, targeted delivery, implantology, immunotherapy, these studies could be helpful to explain the loading and release mechanisms of small-molecule therapeutics in such gels, and in the design of drug delivery devices.

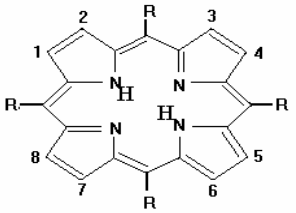
2 Experimental

2.1. Materials

PVA with a polymerization degree of 900 and hydrolysis degree 98% (PVA 90-98), has been provided by the Chemical Plant Risnov.

The porphyrin TSPP (5,10,15,20-tetra-sulfonato-phenyl porphyrin) has been provided by the Institute for Chemical Research ICECHIM. The structure is presented in Fig. 1.

Sodium chloride (NaCl) and potassium chloride (KCl) have been of analytical grade and have been purchased from "Reactivul", Bucharest, Romania.



$R= -C_6H_4-SO_3^-Na^+$

Fig. 1: Structure of TSPP

Potassium dihydrogen phosphate (KH_2PO_4) and Dipotassium hydrogen phosphate (K_2HPO_4) have been of analytical grade and have been purchased from "Reactivul", Bucharest, Romania.

Lactated Ringer's solution has been obtained from Fresenius Kabi GmbH, Bad Homburg, Germany and had the following chemical composition per 1000ml: NaCl 6g, sodium lactate 3,17g, KCl 0,4 g, $CaCl_2 \cdot 2H_2O$ 0,27g, distilled water till 1000 ml. The solution has been used as provided in bottles with rubber septum.

Hydrochloric acid (HCl) and sodium hydroxide (NaOH) have been of have been of analytical grade and have been purchased from "Reactivul", Bucharest, Romania. For pH adjustments, a 1M solution in distilled water has been prepared from both reagents.

2.2. Methods

2.2.1. Preparation of the simple PVA membrane

PVA hydrogel membranes have been prepared by repeated freezing/thawing technique. PVA 90-98 powder has been dissolved, under

magnetic stirring, in distilled water, at 80 ^{0}C, for 3 hours, obtaining a solution with a content in solid matter of 11,04 (%). This solution was cooled and then cast into Petri dishes of 73 mm diameter, with casting thicknesses of 0.1, 0.3, 0.5, 0.7 and 1 mm. Then the PVA solution was submitted to three successive cycles of 12-hour freezing (at -20^{0}C) and 12-hour thawing (at room temperature).

2.2.2. Determination of the content in solid matter of the synthesized gels

For the determination of the content in solid matter, cryogel membrane samples of 2g (both simple and modified with NaCl), have been introduced into weighing phials and kept in a heating oven at 110°C for 10 hours. After the drying period, the phials have been removed from the oven, introduced into an exsiccator and weighed to determine the mass of the obtained xerogel. The content in solid matter has been determined by using the following formula (Eq. 1):

$$CS(\%) = \frac{m_e}{m_x} \quad (1),$$

where m_e represents the mass of the hydrogel that has reached swelling equilibrium in water, m_x stands for the mass of the xerogel obtained after drying.

2.2.3. Preparation of the porphyrin loaded membranes

A 10^{-3} M aqueous TSPP solution has been obtained by dissolving the necessary amount of porphyrin (weighed with an analytical balance – Kern ABJ, Germany, with a precision of 0.1 mg) in distilled water. The porphyrin solution has been kept and handled in the dark due to photosensitivity. The PVA membrane with a casting thickness of 0.5 mm has been loaded by immersing it (30 minutes) into 10 ml of this aqueous TSPP solution.

2.2.4. Preparation of the phosphate-buffered salt solution with pH 7.4

A phosphate-based pH 7.4 buffer has been prepared, with the following composition: K_2HPO_4 47 mM, KH_2PO_4 27 mM, NaCl 137 mM, KCl 27 mM. pH adjustments have been made with 1M solutions of NaOH and HCl in distilled water.

2.2.5. SEM Analysis

For the SEM analysis, the simple PVA hydrogel membrane has been dried by freezing. Additionally, a gold layer has been deposited in order to have sufficient contrast. The porphyrin-loaded membranes have been characterized by SEM in the dried state. For this purpose, after loading with the respective porphyrin, they have been dried for 24 h in a vacuum oven at 25°C.

2.2.6. Characteristic loading and release parameters

Membrane loading has been carried out by immersion into the TSPP solution. The degree of loading (DL%) of the membrane has been calculated according to Eq. 2 and represents the amount of TSPP porphyrin loaded on 1 gram of xerogel.

$$DL(\%) = \frac{(c_{i\_TSPP\_Load} - c_{f\_TSPP\_Load}) \cdot V_{Loading} \cdot M_{TSPP}}{m_{HG\_swollen} \cdot c_{S_{HG\_swollen}}} \cdot 100 \quad (2),$$

where $c_{i\_TSPP\_load}$ is the initial concentration of the TSPP solution used for the loading of the hydrogel membrane, $c_{f\_TSPP\_load}$ is the TSPP concentration at the end of the loading phase, $V_{loading}$ is the volume of TSPP solution used for loading, M_{TSPP} is the molar mass of TSPP, $m_{HG\_swollen}$ is the swollen mass of the hydrogel membrane and $c_{S\_swollen}$ is the content in solid of the swollen hydrogel membrane. The release characteristic has been described using the percentage amount of drug released (DR%), according to Eq. 3.

$$DR(\%) = \frac{c_{TSPP\_released} \cdot V_{release}}{(c_{i\_TSPP\_Load} - c_{f\_TSPP\_Load}) \cdot V_{Loading}} \cdot 100 \quad (3)$$

where $c_{TSPP\_released}$ is the concentration of TSPP in the medium used for release, $c_{i\_TSPP\_load}$, $c_{f\_TSPP\_load}$ and $V_{loading}$ have the same meaning as above, and $V_{release}$ is the volume of the release medium.

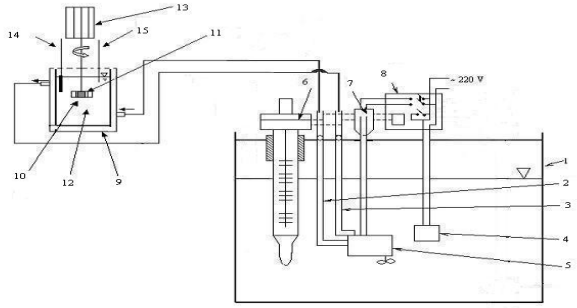
2.2.7. Controlled release study methodology

For the study of the release process, two PVA membrane strips (one simple membrane and a modified membrane) with a mass of 1 g each have been used. In order to avoid possible degradation processes (which could appear if the freezing and thawing method would have been used on the porphyrin-containing PVA solution), loading of TSPP on the pre-formed and conditioned hydrogels (in water) has been preferred. Each PVA membrane (whether simple or modified) has been loaded by immersion into 10 mL of TSPP 10^{-3} M solution for 30 min. Samples have been protected from light exposure at all times during handling and intervessel transfer due to the photosensitivity of the porphyrin. After the loading, the membranes have been taken out of the TSPP solution, rinsed with distilled water, and immersed into 20 mL of release medium, consisting of distilled water, pH 7.4 phosphate buffer or lactated Ringer solution, respectively. Two types of release setups have been used, one in

Erlenmeyer flasks, and one in a scaled-down USP-type basket release apparatus for the controlled release.

For the experiments in Erlenmeyer flasks, he loaded membrane has been placed in the flask containing 20 mL of release medium. The release process has been carried out at room temperature. The porphyrin release has been monitored spectrophotometrically.

The basket release apparatus consists of a motor with controllable agitation rate (in the experiments it has been set at 100 rpm to comply with the USP standard), a basket release setup mounted on a shaft which is rotated by the motor and a double-walled thermostatable dissolution vessel with an effective work volume of 20 ml. The temperature was kept at 37°C with the aid of a thermostat (Ultrathermostat U10, Medingen GmbH, Germany). The apparatus is presented in fig. 2. The temperature in the release medium was monitored with a digital thermometer (Checktemp, Hanna Instruments, USA). The pH value was periodically checked with a digital pH meter (pH 330i, WTW Instruments, Germany). The complete release vessel has been covered with aluminum foil due to the photosensitivity of the porphyrin.



1- thermostat vessel containing water, 2- direct conduit from thermostat, 3- return conduit, 4- electrical resistance for heating, 5- submersible pump, 6- adjustable electrical contact thermometer, 7- motor for driving the pump, 8- control panel, 9- double-walled thermostatable vessel, 10- controlled release basket, 11- hydrogel sample, 12- dissolution medium, 13- vertically mounted electric motor with adjustable rotational speed, 14- digital pH meter with electrode, 15- digital thermometer

Fig. 2. Scheme of the basket-type controlled release apparatus with thermostat

3 Results and Discussion

The problem of mass transfer of porphyrins from PVA-based cryogels (hydrogels synthesized by freeze-thawing) has not undergone any experimental analysis until now in the scientific literature. This is quite surprising, due to the importance of PVA hydrogels as controlled release carriers on the one hand and the applicability of such release mode in PDT, where reduction of side-effects, systemic toxicity and a minimally invasive application is required.

These drug carrier devices can be specifically engineered, in such way that the release process occurs after a certain mass transfer mechanism. Thus, mass transfer can be mediated by Fickian or non-Fickian diffusion, swelling or matrix diffusion (Table 1).

Table 1. Engineering release devices for a particular mass transfer mode

Controlled release device design	**Mass transfer mode**
Membranar hydrogel release device (water-conditioned gel)	Diffusion-mediated release
Xerogel-based release device (dried gel)	Swelling and diffusion- mediated release
Release device based on molecularly imprinted polymer	Matrix diffusion-mediated release

3.1. Gel membrane loading and characterization

The hydrogel membranes and also their TSPP-loaded counterparts have been characterized by SEM. The analysis evidenced an interconnected porous structure of the gel with a pore size distribution of 80-950 nm (Fig. 3). The interconnected pores are good sites for the encapsulation of TSPP, which is visible in form of microgranules on the surface of the PVA gel (Fig. 4).

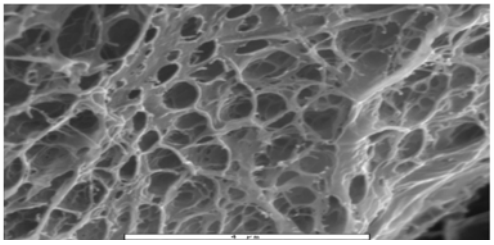


Fig. 3: SEM image of the PVA 90-98 hydrogel

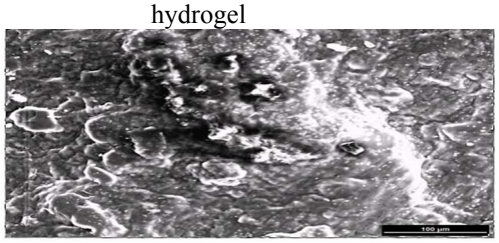


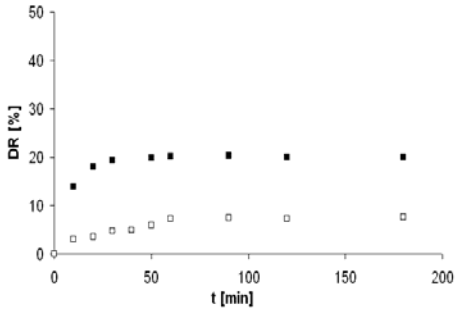
Fig. 4: SEM image of the PVA 90-98 hydrogel loaded with TSPP (dried state).

Thus, according to the SEM analyses, the loading mechanism of the hydrogel is mainly

chemisorption via hydrogen bond formation between the pyrrolic nitrogen of the porphyrin and the hydroxyl group of PVA. A degree of loading with TSPP of 4,6% has been determined. The content in solid of the PVA membrane was c_s=10,71%.

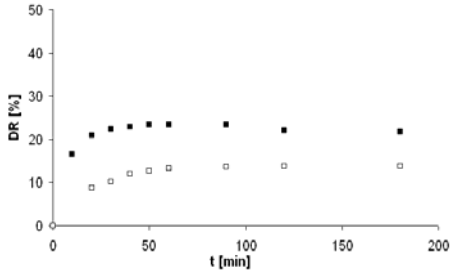
3.2. Influence of agitation on the mass transfer process

We have carried out mass transfer experiments which involve non-agitated vessels (Erlenmeyer flasks) on the one side, and a vertically agitated controlled release apparatus, on the other. The functioning parameters of the agitated vessel (100 rpm, 37°C) comply with USP standards. The experiments were carried out with different dissolution media (PBS buffer and Lactated Ringer's solution, respectively). In both cases, an increase of the transferred amount of porphyrin into the liquid medium has been observed in the case of the vertically agitated vessel (fig. 5 and 6):



■ Agitated vessel □ non-agitated flask

Fig. 5: Temperature influence on mass transfer of TSPP from PVA hydrogel (Release medium: Ringer's solution)



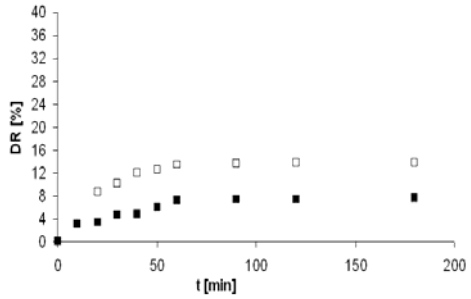
■ Agitated vessel □ non-agitated flask

Fig. 6: Temperature influence on mass transfer of TSPP from PVA hydrogel (Release medium: PBS solution)

This increase can be attributed to the appearance of the convective mass transfer component in the case of the vertically agitated vessel, additionally to the diffusive mass transfer component.

3.3. Influence of release medium on the mass transfer process

The influence of the release medium has also been examined. As can be seen in fig.7, a higher amount of TSPP is transferred into solution when the phosphate-based buffer is used.

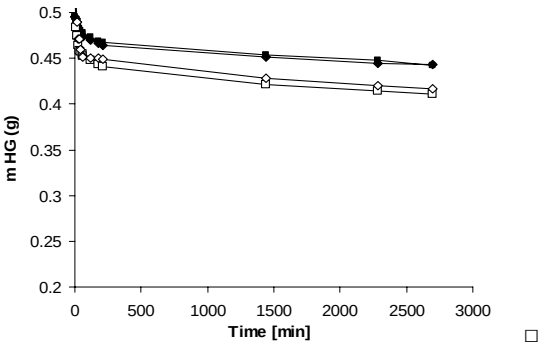


■ Lactated Ringer □ PBS Buffer

Fig.7: Influence of release medium on mass transfer of TSPP at 25°C

In order to provide a mechanistic explanation for this behaviour, we have carried out a collapsation study to examine the behaviour of PVA hydrogel in these media.

The study consisted in immersing hydrogel samples of 0,5 g in 20 mL of each medium and recording the mass changes.



Swollen HG S1, Ringer ◊ Swollen HG S2, Ringer ◆ Swollen HG S1 in PBS ■ Swollen HG S2 in PBS □

Fig. 8. Collapse of swollen PVA cryogel membrane in phosphate buffer and Ringer solution

From the collapsation study (Fig. 8) it can be seen that the PVA cryogel membrane collapses more in the lactated Ringer solution than in the pH 7.4 phosphate buffer. This could explain the results of different amounts of transferred TSPP in these solutions due to cryogel behavior: when compared to the phosphate buffer, the cryogel's pores in lactated Ringer solution (especially the surface pores) become closed in a higher extent, so that

more TSPP is trapped inside the cryogel. This leads to a smaller amount of transferred TSPP in the case of the Ringer solution.

4 Conclusion

The mechanism of mass transfer of a hydrophilic porphyrin-TSPP, encapsulated in PVA-based hydrogels, into various release media, has been examined. Two cases involving diffusion (experiments in non-agitated flasks) and convection-diffusion (experiments in a vertically agitated, thermostated and pH-monitored vessel) have been experimentally investigated. The increase in the transferred amount of the porphyrin in the case of the agitated vessel (when compared with the non-agitated flasks) could be assigned to the convective component of the mass transfer.

Further experimental studies will focus on the determination of diffusion and global mass transfer coefficients and also of effective mass transfer rates for TSPP.

The observations made in this paper are of interest for the future design of controlled release systems with various application routes (implantable, transmucosal or oral routes) for the use in the photodynamic therapy of cancer.

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